show very clearly that the elementary bodies of vaccinia both in the Tiselius apparatus and the analytical centrifuge act in the same way as other particles do. This was demonstrated by making collodion and glass particles of the same size as elementary bodies, coating them with various proteins, including specific ones from vaccinia, and investigating their behavior in the two apparatuses when mixed with elementary bodies of vaccinia.

During the course of the chemical studies of vaccinia made by Dr. Hoagland it was necessary to devise micromethods for the accurate estimation of certain substances. As a result of this necessity micromethods are now available for the determination of phosphate, sulfate and magnesium. Only 0.5 microgram of phosphorus is required for an accurate determination. As little as 20 micrograms of sulfate can now be determined accurately. Ten micrograms of magnesium yield an error less than 2 per cent with the new method.

PNEUMONIA

Dr. Avery and Associates.

Dr. Avery and his associates have continued their work on pneumonia and the pneumococcus. Some of the work described in this report is a continuation of that reported last year, at which time it was noted that drugs of the sulfonamide group had to a large extent replaced the use of antipneumococcus serum. It has been previously shown that drug-fast (resistant to the drug) strains of pneumococci can be developed in the laboratory. Inasmuch as all patients receiving sulfonamide drugs do not recover and since some recover more promptly than others, it was thought advisable to determine whether there is a correlation between clinical results in the use of drugs and degrees of drug fastness in the organisms isolated from the patients at different times in the course of the disease. Twenty-two patients with pneumonia were investigated in this manner. A comparison of the results obtained in these patients showed that there is a correlation. Further studies are planned to determine the conditions under which pneumococci develop resistance to the sulfonamide drugs and the nature of the alterations brought about in the microorganisms when this phenomenon occurs.

In last year's report it was stated that in certain materials, particularly in pus, there are inhibitors to the bacteriostatic (power to prevent growth) action of the sulfonamide drugs and that one of the inhibitors had been isolated and partially purified. In the meantime, reports from England have stated that p-aminobenzoic acid acts as a powerful sulfonamide inhibitor. Workers in Dr. Avery's laboratory in attempting to correlate these findings with their own and to determine as accurately as possible whether p-aminobenzoic acid alone is responsible for all inhibition, have carried on work similar to that of Dr. Dubos in the development of adaptive enzymes. As a result of these efforts, Dr. Mirick has obtained from a soil bacillus an enzyme which will more or less specifically modify p-aminobenzoic acid so that it will no longer act as a sulfonamide inhibitor. The use of this adaptive enzyme has clearly shown that some inhibitors are of the general nature of p-aminobenzoic acid, while others certainly are not. This enzyme obviously affords a very useful tool for the further study of the nature and action of the sulfonamide inhibitors and for the detection of minute amounts of p-aminobenzoic acid.

Since the introduction of the sulfonamide drugs into the armamentarium of the physician, arguments regarding how these chemical agents effect a cure have arisen. Some contend that the drugs have a direct action on the pneumococcus, while others hold that immunological forces actually bring about the cure, eliminating pneumococci held in check by the bacteriostatic action of the drugs. This latter view is held by Dr. Avery and his associates, and during the year certain experimental findings in rabbits give further support to the idea that in man effective sulfonamide drug therapy is intimately associated with the development of active immunity.

The work of Dr. Dubos and Dr. Hotchkiss concerning bactericidal substances obtained from a soil bacillus (B. brevis) has kept the Hospital in the forefront in the field of chemotherapy. Remarks concerning these activities were made last year; since then the work has continued actively and two substances, gramicidin and the hydrochloride of tyrocidine, have been obtained in crystalline and in highly purified form. Both of these substances are polypeptides; the former is active against all Gram-positive bacteria so far tested except the tubercle bacillus, while the latter is active against both Gram-positive and Gram-negative bacteria. Further work is in progress to ascertain fuller knowledge concerning the structure of these two substances and the manner in which they act to kill microorganisms. Early in the work it was found that tyrocidine would not act in vivo (in the living body), and that gramicidin had a limited action in vivo, particularly against blood-stream infections. Because of the toxicity of these compounds and their limited value under certain conditions, they have not been employed in the treatment of pneumonia in human beings. Recently an explanation has been found of why gramicidin probably is ineffective against Gram-negative organisms and why it is impotent against Gram-positive organisms in the blood stream. Dr. Folch-Pi on Dr.

Van Slyke's service has discovered a new type of cephalin (belonging to the family of fats) which is now known as "amino acid cephalin" and has demonstrated the presence of this substance in the blood stream. During the course of some work Dr. Dubos and Dr. Coburn showed that the "amino acid cephalin" strongly inhibits the action of gramicidin. It is also known that Gram-negative organisms, against which gramicidin is impotent, contain a polypeptide-polysaccharide-phospholipid complex. This complex obtained from dysentery organisms and colon bacilli is very active in inhibiting the effect of gramicidin on microorganisms. Whether this inhibitory action is due to the presence of the phospholipid cephalin in the complex removed from the Gram-negative organisms cannot be considered as established, but it is interesting to note that the cellular structure which remains after removal of the complex is without effect upon the action of gramicidin.

A number of years ago Dr. Avery and his associates found in the blood of patients acutely ill with pneumonia a protein which was designated as C-reactive (C is a sugar found in bodies of pneumococci) protein and which is not present in the blood of normal individuals. The protein is an albumin -- this differentiates it from ordinary antibodies which are globulin -- and is demonstrable by precipitation when it is mixed with the C-polysaccharide obtained from pneumococci. Later it was found that C-protein occurs not only in pneumonia patients, but in the blood of individuals suffering from a number of acute and chronic infections. In addition, they showed that a specific antiserum can be made against the C-protein and that through the use of this antiserum much smaller quantities of it can be detected than by means of the precipitation reaction in which the C-polysaccharide is employed. During the past year, using an antiserum against the C-protein for its detection in the serum of patients, workers in Dr. Avery's laboratory were able to show that there is a closer correlation between the presence of C-protein and the clinical condition of the patient than there is between the clinical condition of the patient and the white blood cell count or the sedimentation rate of the red blood cells. All of this may sound very technical, but it means that there has been discovered in the blood serum of patients suffering from infectious diseases another substance which was previously unknown and which is not demonstrable in the blood of normal individuals. This discovery offers another tool for the study of what goes on in the body of a person suffering from an infection, something in which all research physicians are greatly interested.

The capacity of pneumococci to grow and produce disease in the animal body is conditioned by, if not wholly dependent upon, the activity of the particular group of enzymes concerned in the synthesis of the cell capsule. Capsular synthesis is most highly developed and the product of its activity most pronounced in cells best adapted to growth in the animal body. The presence in the capsule of a chemically unique and serologically reactive polysaccharide (complex sugar) confers upon the cell a highly selective specificity which makes possible the differentiation of sharply defined and specific types within the species. The enzymes responsible for capsular synthesis can be reversibly inactivated by known changes in environmental conditions without impairing the viability of the microorganisms. The selective inactivation of this particular function results in the loss of capsules, together with the consequent loss of type specificity and disease-producing properties. Under these conditions highly pathogenic microorganisms are reduced to a state in which they are no longer capable of inducing disease in animals highly susceptible to fatal infection with the originally encapsulated parent strain.

Important and essential as capsular synthesis is to the disease-producing properties of pneumococci in the living host, this function is not vital to the life and growth of the microorganisms outside the animal body, since cells in which the formation of capsules has been inhibited are still capable of carrying on the vegetative processes of metabolism and multiplication in artificial media. Capsule formation may be regarded, therefore, as an adaptive mechanism whereby the bacterial cell seeks to protect itself against the defense reactions of the host.

For a number of years it has been known that by experimental means a pneumococcus that is virulent and surrounded by a capsule made up of complex sugars can be induced to lose its capsule and become avirulent. Furthermore, it has been possible by the proper means to induce a Type I Pneumococcus to become a Type III Pneumococcus. Much of this work has been done in Dr. Avery's laboratory. He has always been interested in the transformation of one type of pneumococcus into another and from time to time has sought, along with his associates, the substance responsible for this transformation. Progress has been made gradually, and within the last year Dr. MacLeod and Dr. Avery have been able to obtain from cell-free extracts of encapsulated pneumococci a substance which seems to be an ester, and which in very small quantities is active in bringing about transformation of one type of pneumococcus to another.

The study is being continued with the hope that knowledge of this important cellular mechanism may lead to a better understanding of the principles involved in certain induced variations of living cells, not only of the pneumococcus, but also those of other biological systems. Furthermore, it is possible that knowledge pertaining to the nature of the substances which serve as activators and inhibitors of the capsule-producing enzymes might afford a specific approach to the suppression of the capsular function, upon the activity of which the disease-producing properties of the pneumococcus depends. Such a statement might be interpreted as dealing with a new approach to the prevention and cure of pneumonia.

For a number of years Dr. Goebel and his associates have been studying the chemical structure of the complex sugars that make up the capsules of pneumococci. In a previous report it was stated that the structure of the Type III polysaccharide had been determined. Dr. Goebel was not satisfied with the evidence and proceeded to obtain confirmation of his findings by a different chemical approach. This work has been completed and his previous findings have been fully substantiated, and now the structure of at least one of the complex capsular sugars of the pneumococcus is firmly established.

In 1911 Forssman showed that in certain animal tissues, for instance horse kidney, a substance or antigen occurs which when injected into rabbits produces antibodies that agglutinate sheep red cells. This antigen has come to be known as the Forssman or heterophile antigen. Some years after this discovery, it was shown that pneumococcus cells and certain other bacteria contain the antigen. Inasmuch as the workers in Dr. Avery's laboratory have always been interested in studying all of the components of the pneumococcus, Dr. Goebel undertook further investigation of the heterophile antigen of this organism and has been able to obtain it in a relatively pure state. Early in the work he had difficulty in separating the antigen from the complex sugar of the body of the pneumococcus which has been designated as C-polysaccharide. He finally devised a method of separating the two substances and now believes that the Forssman antigen is a complex sugar somewhat similar to the C-polysaccharide, yet differing from the latter in that it contains a chemical grouping not possessed by the former. Further work will be carried on with this substance in the hope of obtaining a better understanding of the pneumococcus cell.

RHEUMATIC FEVER

Dr. Swift and Associates.

Dr. Swift and his associates have continued their investigations of the relation of homolytic streptococci to rheumatic fever. In last year's report it was stated that attempts had been made to confirm observations of British workers to the effect that hemolytic streptococci are obtained at autopsy from heart valves injured by rheumatic fever. The evidence at that time seemed fairly conclusive that the British observers were in error and that the streptococci obtained by them were not of etiological significance but contaminants encountered because of a lack of sufficient aseptic technique in the performance of the autopsies. This work has been continued with an improvement in the technique of performing autopsies; in fact, Dr. Swift and his associates now employ strict surgical technique in the performance of autopsies. The results of this additional work substantiate their previous findings, because in the presence of proper technique hemolytic streptococci have not been found in rheumatic heart valves.

The negative findings just mentioned do not invalidate the conception that the hemolytic streptococcus is the cause of rheumatic fever. In fact, most workers recognize that this organism is in some way associated with the disease. However, the nature of the association is not clear. In order to throw light upon this problem, Dr. Swift and his asso-